JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Antioxidant Activity of Phenolic Compounds Isolated from *Mesona procumbens* Hemsl.

CHIEN-YA HUNG AND GOW-CHIN YEN*

Department of Food Science, National Chung Hsing University, 250 Kuokuang Road, Taichung 402, Taiwan, Republic of China

The antioxidant activity of phenolic compounds isolated from *Mesona procumbens* Hemsl. (Hsian-tsao) was investigated. Hsian-tsao was extracted with various solvents, and the results showed that the fraction treated with acidic ethyl acetate (pH 2) possessed large amounts of phenolic compounds and a strong antioxidant activity on peroxidation of linoleic acid. The antioxidant activity (inhibition of peroxidation, IP%) of the acidic ethyl acetate of Hsian-tsao extract at 50 μg/mL (98.9%) was stronger than those of 50 μg/mL α-tocopherol (78%) and BHA at 10 μg/mL (90%). When fractionated with Amberlite XAD-7 gel chromatography, the acidic ethyl acetate fraction of Hsian-tsao extract was separated into four subfractions (A−D). Subfraction B, with high yield and strong antioxidant activity, was further isolated and purified and then identified as containing protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, and syringic acid by means of UV, EI-MS, and ^{1}H and ^{13}C NMR. The antioxidant capability of isolated compounds was also determined using the thiocyanate system and the erythrocyte ghost system. The results indicate that the phenolic acids could be important antioxidant components in Hsian-tsao, among which caffeic acid with the highest antioxidant activity and the greatest content is most important.

KEYWORDS: ***Mesona procumbens*** **Hemsl. (Hsian-tsao); antioxidant activity; phenolic acid; erythrocyte ghost system**

INTRODUCTION

Phenolic compounds are naturally occurring substances in fruit, vegetables, nuts, seeds, flowers, and some herb beverages and are an integral part of the human diet. Several studies have indicated that the antioxidant activities of some fruits and vegetables were highly correlated to their total phenolic contents (*1*, *2*). Hertog et al. (*3*) found an epidemiological link between the intake of polyphenols and the risk of cardiovascular disease in humans. Several researchers have also shown evidence of in vivo antioxidant effects after ingestion of polyphenol-rich beverages, such as tea and wine (*4*, *5*). Therefore, an investigation of such antioxidative phenolic compounds in edible plants has been conducted to improve our understanding of their dietary value and potential benefits.

The herb *Mesona procumbens* Hemsl., called Hsian-tsao in China, has been consumed as a popular herbal drink and a gelatin-type dessert in the Orient. It has also been used as a folk medicine to treat heat-shock, hypertension, diabetes, and liver diseases. Many compounds, such as sterol compounds, stigmasterol, β-sitosterol, triterpene compounds, oleanolic acid, and ursolic acid, have been isolated from Hsian-tsao (*6*). Related studies have indicated that oleanolic acid and ursolic acid showed many biological effects, including hypoglycemia, antiinflammatory and hepatoprotective effects, and relief of acute and chronic hepatitis (*6*, *7*). Balanehru and Nagarajan (*8*) reported that oleanolic acid and ursolic acid could protect lipid peroxidation of liver microsomes induced by free radicals. Kazuki et al. (*9*) reported that the water extract of Hsian-tsao had high antioxidative and free radical scavenging abilities. Moreover, the addition of the water extract of Hsian-tsao in beverages reduced the oxidation of ascorbic acid in the drinks.

In our previous study, we found that phenolic compounds extracted from Hsian-tsao significantly contributed to the antioxidant activity and free radical scavenging effects (*10*). However, the major phenolic components related to the antioxidant effects were unclear. The purpose of this study was to evaluate the antioxidant activities of compounds isolated from Hsian-tsao extract with different systems, including the thiocyanate method and the erythrocyte ghost system.

MATERIALS AND METHODS

Chemicals. 2,6-Dichloroindophenol (DIP), caffeic acid, thiobarbituric acid, and disodium hydrogen phosphate were purchased from E. Merck (Darmstadt, Germany). BHA, linoleic acid, α-tocopherol, β-carotene, gallic acid, phenylmethanesulfonyl fluoride (PMSF), ascorbic acid, and Amberlite XAD-7 resin were purchased from the Sigma Chemical Co. (St. Louis, MO). Ammonium thiocyanate, potassium hydroxide, sodium alginate, pyrogallol, and Folin−Ciocalteu reagent were purchased from the Wako Pure Chemical Co. (Tokyo, Japan).

* Author to whom correspondence should be addressed (fax 886-4-2285-4378; e-mail gcyen@mail.nchu.edu.tw).

10.1021/jf011454y CCC: $22.00
Published on Web 04/11/2002

6-Hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) was purchased from the Aldrich Chemical Co. (Milwaukee, WI).

General Procedures. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian VXR-300S FT-NMR spectrometer (Harbor City, CA) operated at 300 MHz for ^{1}H NMR and at 75.43 MHz for ^{13}C NMR with complete proton decoupling. The spectra were observed on CD_3OD. Chemical shifts were recorded as δ values using tetramethylsilane as an internal standard. The electron impact mass spectra (EI-MS) were recorded on a JEOL JMS-SX/SX 102A mass spectrometer (JEOL Co.). The UV−vis absorption spectra of the active components in solution were recorded on a spectrophotometer (Hitachi U-2000).

Sample Preparation. Dried Hsian-tsao (*Mesona procumbens* Hemsl.) harvested in 1995 was purchased in Hualian, Taiwan. The sample was ground into a fine powder with a mill (RT-08, Rong Tsong, Taichung, Taiwan). The powder was passed through an 80-mesh sieve, collected and sealed in a plastic bag, and then stored at 0−4 °C for further use.

Preparation of Hsian-tsao Extracts and Fractions. The liquid−liquid extraction method was used to obtain several fractions containing different classes of polyphenolic compounds. Five kilograms of Hsian-tsao powder was extracted three times with 75% methanol (15 L) for 24 h according to the method described by Conde et al. (*11*) with a slight modification. The extract was filtered with Whatman No. 1 filter paper and evaporated on a vacuum rotary evaporator (<4°C) under reduced pressure to remove the methanol. The aqueous solution was successively partitioned using *n*-hexane, benzene, ethyl acetate, acidic ethyl acetate (pH 2), and *n*-butanol. Each fraction was then evaporated in vacuo until dry and weighed to determine the yield, antioxidant activity, and total phenolic compounds.

Isolation and Identification of the Antioxidants from Hsian-tsao Extracts. Five grams of dried acidic ethyl acetate fraction of Hsian-tsao was introduced on top of an Amberlite XAD-7 column (100 × 6.6 cm). The column was eluted with methanol/water/acetic acid (70:29.9:0.1, v/v/v) solution and 100% methanol. Eluents of each 50 mL were collected, and the absorbance of each was measured at 280 nm using a spectrophotometer (Hitachi U-2000). Four subfractions (fractions A−D) were found. The yield and antioxidant activity of each subfraction were determined using the method described above. The yields of subfractions A, B, C, and D were 0.160, 3.259, 1.068, and 0.607 g, respectively.

Purification of subfraction B, which exhibited the strongest antioxidant activity among the four subfractions, was carried out using a preparative HPLC equipped with a Develosil ODS 10 reversed phase column (250 × 20 mm i.d., Nomura Chemical Co.) and a UV−vis detector (Hitachi Ltd.). The column was eluted with methanol/water/acetic acid solution (10:89:1 to 20:79:1, v/v/v) at 5.0 mL/min. Five compounds were isolated and identified as protocatechuic acid (**1**, t_R = 17.74 min, 11.7 mg), *p*-hydroxybenzoic acid (**2**, t_R = 34.05 min, 19.8 mg), vanillic acid (***3***, t_R = 56.67 min, 20.3 mg), caffeic acid (**4**, t_R = 72.51 min, 80.4 mg), and syringic acid (**5**, t_R = 92.56 min, 6.8 mg) by means of UV, EI-MS, and ^{1}H and ^{13}C NMR spectrometry. The antioxidant activity of each compound (1 mg) dissolved in 1 mL of methanol was determined using the thiocyanate method (*12*).

Determination of Total Phenolics. The concentrations of total phenolics in Hsian-tsao extracts were measured using the Folin−Ciocalteu assay (*10*). A sample (0.1 mg) was dissolved in a test tube with 0.1 mL of distilled waterr; 50% Folin−Ciocalteu reagent (0.1 mL) was added and mixed in thoroughly. After an interval of 3 min, 2 mL of 2% Na_2CO_3 solution was added, and the mixture was allowed to stand for 30 min with intermittent mixing. The absorbance of the mixture at 750 nm was measured on a Hitachi spectrophotometer (model U-2000). A standard curve using gallic acid was also prepared. Results were expressed as milligrams per gram of extract of gallic acid equivalents (GAE).

Determination of Ascorbic Acid. The ascorbic acid was determined using the 2,6-dichloroindophenol (DIP) method with a modification (*13*). One milligram of water extract of Hsian-tsao was dissolved in 1 mL of 1% metaphosphoric acid and filtered with Whatman No. 1 filter paper. Nine milliliters of 50 μM DIP was added to 1 mL of extract and incubated at room temperature for 15 s. The developed color was measured at 515 nm using a Hitachi U-2000 model spectrophotometer. The concentration of the ascorbic acid in the Hsian-tsao extract was determined through comparison with the absorbance of standard ascorbic acid at different concentrations.

HPLC Analysis of Tocopherol Isomers and β-Carotene. Tocopherol isomers were extracted using the method of Yen and Chen (*13*). Hsian-tsao water extract (1 mg) was mixed with pyrogallol (6 mL, 6%) and potassium hydroxide (4 mL, 60%), and the mixtures were incubated at 70 °C for 20 min. A portion (15 mL) of distilled water was added to the mixture, which was then extracted with *n*-hexane (15 mL). The hexane layer was dried over Na_2SO_4 and then evaporated to dryness. The resultant residue was redissolved in 5 mL of *n*-hexane and separated on HPLC. Tocopherols were determined in a LiChrosorb Si-60 column at 295 nm and with a mobile phase of *n*-hexane/2-propanol/ethanol (100:3:2, v/v/v) at a flow rate of 1.0 mL/min.

β-Carotene was extracted using the method of Kitada et al. (*14*). Hsian-tsao water extract (0.1 g) was mixed with 10 mL of 1% pyrogallol in methanol/dichloromethane (1:1, v/v). The mixture was filtered through a 0.45 μm filter and injected into the HPLC. Analysis of β-carotene was conducted using HPLC with a UV−vis detector (470 nm) and a LiChrosphere RP-18 column (250 × 4 mm, 5 μm). The column was equilibrated with acetone/methanol/acetonitrile (1:2:2, v/v/v) at a flow rate of 0.7 mL/min. All of the analyses were conducted in three replicates, and the results were averaged.

Determination of Antioxidant Activities Using the Thiocyanate Method. The antioxidant activities of Hsian-tsao extracts and solvent-fractionated compounds were determined using the thiocyanate method (*12*). The extracts and fractionated compounds (final concentration = 50 μg/mL, dissolved in water), antioxidants for BHA (final concentration = 10 μg/mL, dissolved in methanol) and α-tocopherol (final concentration 50 μg/mL, dissolved in methanol), were added at 0.5 mL into a test tube containing a mixture of linoleic acid (2.5 mL, 0.02 M) and potassium phosphate buffer (2 mL, 0.2 M, pH 7.0). The mixture was incubated at 37 °C for 72 h. At regular intervals, an aliquot was taken and reacted with ferrous chloride and thiocyanate. The absorbance (*A*) of the reactant at 500 nm was measured as peroxide value after the reaction. A blank was also prepared with distilled water. Each sample was run in triplicate. The percentage of inhibition of peroxidation (IP%) (the capacity to inhibit peroxide formation in linoleic acid at 72 h) was calculated as follows: $IP\% = [1 - (A_{sample}/A_{control})] \times 100$.

Erythrocyte Ghost System of Antioxidant Compounds from Hsian-tsao. Erythrocyte ghost membranes were prepared from human blood cells using the method of Miyake et al. (*15*) with modification. Human blood (100 mL) was collected from healthy individuals, using heparin sulfate to prevent clotting, and diluted with 400 mL of PBS (5 mM phosphate-buffered saline/1 mM EDTA, 0.5 mM PMSF, pH 7.4) overnight. The diluted blood solution was centrifuged at 1500*g* for 20 min, and the erythrocyte was collected and washed three times with PBS. Erythrocyte ghost membranes were suspended in PBS containing 0.5 mM PMSF at a concentration of 1 mg of protein/mL.

Erythrocyte ghost membranes were oxidized by incubation in the presence of Fe^{2+} ion. Phenolic compounds (0.1 mL) were dissolved in distilled water, and the sample solution was incubated with the ghost suspension (0.5 mL), 0.1 mL of 5 mM $FeSO_4$, 0.1 mL of 10 mM KCl, 0.1 mL of 0.05 mM ascorbic acid, and 0.1 mL of PBS. After incubation at 37 °C for 2 h, 0.5 mL of 10% HCl and 0.5 mL of 1% thiobarbituric acid (TBA) solution were added to stop the reaction. The quantity of TBA reactive substance (TBARS) was determined at 532 nm. A control containing no added sample represented 100% lipid peroxidation.

Statistical Analysis. All results were obtained from three independent experiments and averaged. Statistical analyses were performed according to the *SAS User's Guide*. Analyses of variance were performed using the ANOVA procedure. Significant differences between the means were determined using Duncan's multiple-range test.

RESULTS AND DISCUSSION

Antioxidant Compounds in Water Extract of Hsian-tsao. The content of total phenolics, ascorbic acid, tocopherols, and β-carotene in the water extract of Hsian-tsao was investigated, among which the total phenolics had the highest content of 185.4

Table 1. Yields, Ratios, and Total Phenolic Contents of Various Solvent-Extracted Fractions from the 75% Methanol Extract of Hsian-tsao

fraction	yield[a] (mg/g of Hsian-tsao extract)	ratio[b] (%)	phenolic content (mg/mg of extract)
n-hexane	81.16 ± 25.09	8.1	0.05 ± 0.01
benzene	55.05 ± 7.44	5.5	0.07 ± 0.01
ethyl acetate	58.55 ± 2.54	5.9	0.16 ± 0.03
acidic ethyl acetate (pH 2)	26.27 ± 4.15	2.6	0.46 ± 0.07
n-butanol	157.61 ± 13.18	15.8	0.15 ± 0.02
residual water layer	621.38 ± 60.65	62.1	0.07 ± 0.03

[a] Hsian-tsao powder (100 g) was extracted with 75% methanol (2 L) three times at room temperature for 24 h, and the filtrate was evaporated to remove methanol. The concentrate was successively fractionated with *n*-hexane, benzene, ethyl acetate, acidic ethyl acetate (pH 2), and *n*-butanol. Values are means of three replicate analyses. [b] Ratio (%) = solvent extract/75% methanol extract.

mg/g of extract. The contents of ascorbic acid and tocopherols were 5.96 and 0.33 mg/g of extract, respectively. β-Carotene was not detected in the water extract of Hsian-tsao. Azuma et al. (*16*) reported that the antioxidant activity of ascorbic acid (257.8 mg/100 g of fresh weight) was found only during the initial period of peroxidation, and the tocopherols (14.0 mg/100 g of fresh weight) were found at much lower levels than phenolic compounds in *Corchorus olitorius* L. Therefore, ascorbic acid and tocopherols may not be the primary antioxidant components in the water extract of Hsian-tsao. The antioxidant activity and the scavenging effects of the Hsian-tsao water extract were highly correlated ($r^2 = 0.85$) with the total phenolic contents (*10*). We suggest that the phenolic compound could be the predominant antioxidant in the Hsian-tsao water extract. Hence, in this work, the isolation, identification, and efficacy of antioxidant polyphenolic compounds from the Hsian-tsao water extract are investigated.

Antioxidant Activities by Thiocyanate Method and Total Phenolic Compounds of Fractions from Hsian-tsao. Hsian-tsao extracts obtained using 75% methanol were rich in the content of phenolic compounds. **Table 1** shows the yields, percentages, and total phenolic compounds of various solvent-extracted fractions from the 75% methanol extract of Hsian-tsao. Among the various fractions, the residual of the water layer had the highest yields, and the acidic ethyl acetate (pH 2) fraction had the lowest.

Antioxidant activities of various solvent fractions were compared with those of BHA and α-tocopherol. **Figure 1** shows that fractions extracted with acidic ethyl acetate (pH 2), ethyl acetate, *n*-butanol, and benzene exhibited significant antioxidant activities. The antioxidant activity of BHA was 90% at the concentration of 10 μg/mL, and that of α-tocopherol was 78% at 50 μg/mL. The antioxidant activities of these fractions at a concentration of 50 μg/mL were higher than that of BHA (90%) at 10 μg/mL and significantly higher than that of α-tocopherol (78%) at 50 μg/mL ($p < 0.05$), especially the acidic ethyl acetate (pH 2) fraction, which exhibited 98.9% inhibition of peroxidation on linoleic acid.

As shown in **Table 1** the acidic ethyl acetate (pH 2) fraction had the highest contents of phenolic compounds. This result was similar to those of previous studies (*10*), which showed that the contents of phenolic compounds could contribute significantly to the antioxidant activity. Many investigations that focused on fruits, vegetables, Du-zhong (*Eucommia ulmoides*), and plant extracts found positive and highly significant relationships between total phenolic contents and antioxidant activity

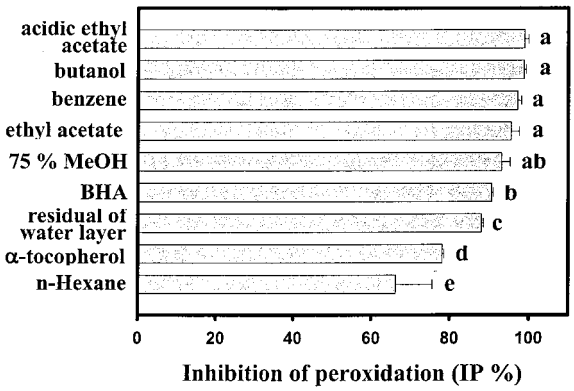

Figure 1. Antioxidant activity of solvent-extracted fractions from Hsian-tsao extracts as measured by the thiocyanate method. The concentrations of BHA and α-tocopherol were 10 and 50 μg/mL, respectively, and the concentration of solvent-extracted fractions was 50 μg/mL. Different letters indicate significant difference ($p < 0.05$). Each value is the mean ± standard derivation of three replicate analyses.

(*1*, *17*−*19*). Hence, this study focused on the purification of the acidic ethyl acetate fraction, which had a stronger antioxidant activity and higher contents of phenolic compounds.

To discover the constituents of the phenolic compounds of the acidic ethyl acetate fraction of Hsian-tsao, the fraction was fractionated into four subfractions (A−D). The antioxidant activities of the subfractions at the same concentration, 50 μg/mL, were in the following order: BHA (10 μg/mL) > fraction B (50 μg/mL) > fraction E (50 μg/mL) > fraction A (50 μg/mL) > α-tocopherol (50 μg/mL) > fraction C (50 μg/mL). Using preparative HPLC, purification of the subfraction of B, which exhibited the strongest antioxidant activity and highest yields among the four subfractions, was carried. From UV−vis, EI-MS, and ^{1}H and ^{13}C NMR data, five purified compounds were identified as protocatechuic acid (**1**), β-hydroxybenzoic acid (**2**), vanillic acid (**3**), caffeic acid (**4**), and syringic acid (**5**). The spectral characteristics of compounds **1**−**5** were identical to those reported by Zhang et al. (*20*), Kuo and Shue (*21*), Kuo and Shue (*21*) and Sakushima et al. (*22*), Uchida et al. (*23*), and Chen et al. (*24*) and Inoshiri et al. (*25*), respectively. The structures of the five compounds are shown in **Figure 2**.

Antioxidant Activity of Purified Compounds. *Linoleic Acid Peroxidation.* **Figure 3** shows a comparison of the antioxidant activities of compounds **1**−**5** with the antioxidants α-tocopherol, Trolox, BHA, and the antioxidant compounds of Hsian-tsao, oleanolic acid, and ursolic acid, at the same concentration of 200 μg/mL. The results showed that the antioxidant activity of caffeic acid was stronger than that of the others. The antioxidant activities of the isolated compounds were in the following order: BHA = Trolox > 75% methanol extract of Hsian-tsao > caffeic acid (**4**) > α-tocopherol > protocatechuic acid (**1**) > syringic acid (**5**) > vanillic acid (**3**) > *p*-hydroxybenzoic acid (**2**). Several studies have shown that caffeic acid is a very potent antioxidant in different systems (*26*, *27*), with efficiency close to or even greater than that of BHT (*28*). However, the antioxidant activity is related to the structure, in particular to electron delocalization of the aromatic nucleus present in phenolic compounds. Pokorny (*29*) and Cuvelier et al. (*30*) indicated that the presence of the −CH═CH−COOH group in cinnamic acid ensured greater antioxidant activity than did the presence of the COOH group in benzoic acid. This may suggest that the C═C double bond participates in stabilizing the radical by means of resonance. On the other hand, the fact that benzoic acid was found to be less efficient than cinnamic acid tends to

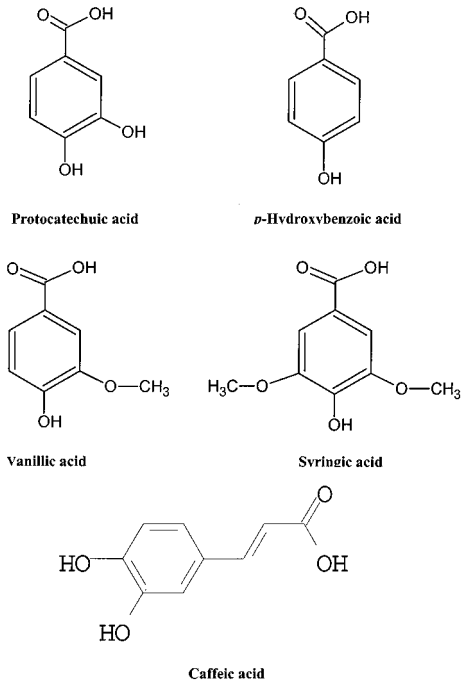

Figure 2. Structures of compounds isolated from *M. procumbens*.

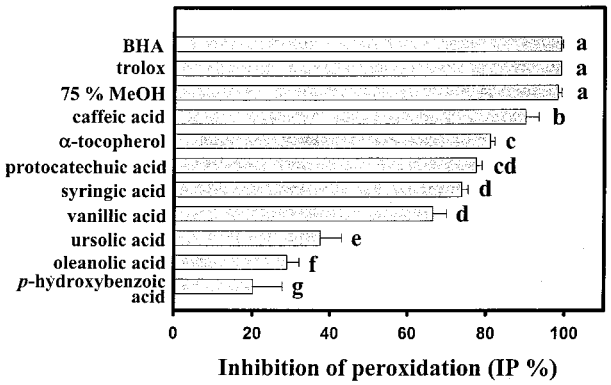

Figure 3. Comparison of antioxidant activity of isolated compounds and Hsian-tsao water extract. The activity was determined by the thiocyanate method. The concentration of BHA, α-tocopherol, Trolox, water extract of Hsian-tsao, and isolated compounds was 200 μg/mL. Different letters indicate significant difference ($p < 0.05$). Each value is the mean ± standard derivation of three replicate analyses.

support a negative role for the COOH group (*30*). This is in agreement with the results of our study.

Erythrocyte Ghost System. The biological membranes, particularly in cell, mitochondria, and erythrocytes, are considered to be critical targets for cell damage. An erythrocyte ghost was usually used as the target for biological damage. In this study, the inhibitory effect of Hsian-tsao on lipid peroxidation of erythrocytes membrane was investigated. Caffeic acid and protocatechuic acid also showed higher activity in the erythrocyte ghost system assay (**Figure 4**). On the basis of the structural characteristics, it was concluded that the *o*-dihydroxy groups on the benzene rings could increase the levels of antioxidant activity, and it could be the major mechanism caused by suppression of chelating metal ions. Thus, the low protective effects of vanillic and syringic acid on the erythrocyte ghost system were caused by methoxylation of the hydroxyl groups, which resulted in a rapid decrease in antioxidant activity due to loss of the chelating capability. Miyake et al. (*15*) and Sanbongi et al. (*31*) reported that the aglycons of flavonoid

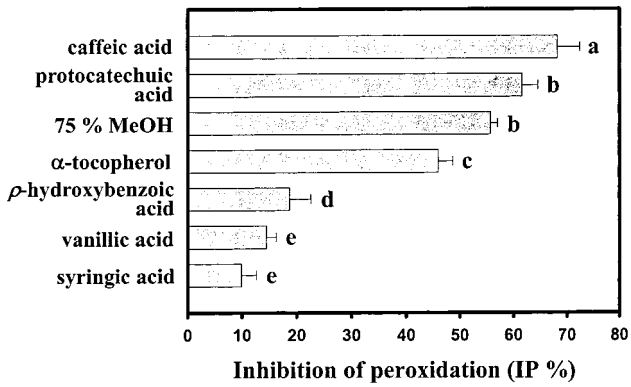

Figure 4. Inhibitory effect of water extract of Hsian-tsao, phenolic compounds, and α-tocopherol on iron(II)-initiated oxidation of the human erythrocyte membrane ghost system. The concentration of sample was 100 μg/mL. Different letters indicate significant difference ($p < 0.05$). Each value is the mean ± standard derivation of three replicate analyses.

glycosides containing adjacent dihydroxy groups on the B ring showed high antioxidant activity in the erythrocyte ghost system. Ohnishi et al. (*32*) reported that caffeic acid and chlorogenic acid inhibited the oxidation of the erythrocyte ghost induced by H_2O_2. The data suggest that caffeic acid may act as a strong antioxidant in Hsian-tsao and possesses a protective capability in the biological membrane system.

In this study, five phenlolic compounds, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, and syringic acid, were isolated from Hsian-tsao extracts. Results showed that the phenolic compounds played important antioxidative roles in Hsian-tsao extracts and that caffeic acid had high yield and antioxidant activity. This finding may in part explain some of the suggested health benefits of beverages made with Hsian-tsao. If the antioxidant activity of Hsian-tsao was calculated as 100%, the total antioxidant efficiencies of the phenolic antioxidant in the extract for caffeic acid were 9.1 and 12.2% in thiocyanate method and erythrocyte ghost system, respectively. These results mean that the Hsian-tsao extract might contain other unknown antioxidant compounds. In our previous study, some compounds with low antioxidant activity, such as ursolic acid and oleanolic acid, have been isolated from Hsian-tsao (*33*). The results suggest that the antioxidant activity of Hsian-tsao should have the other antioxidant compounds and/or may express a synergistic effect. However, further study is needed.

LITERATURE CITED

(1) Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113−4117.

(2) Emmons, C. L.; Peterson, D. M.; Paul, G. L. Antioxidant capacity of oat (*Avena sativa* L.) extracts. 2. In vitro antioxidant activity and contents of phenolic and tocopherol antioxidants. *J. Agric. Food Chem.* **1999**, *47*, 4894−4898.

(3) Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, O. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* **1993**, *342*, 1007−1011.

(4) Whitehead, T. P.; Robinson, D.; Allaway, S.; Syme, J.; Hale, A. Effect of red wine ingestion on the antioxidant capacity of serum. *Clin. Chem.* **1995**, *41*, 32−35.

(5) Deng, Z.; Tao, B.; Li, X.; He, J.; Chen, Y. Effect of green tea and black tea on the blood glucose, the blood triglycerides, and antioxidation in aged rats. *J. Agric. Food Chem.* **1998**, *46*, 3875−3878.

(6) Sheu, S. Y.; Liu, C.; Chiang, H. C. The hypoglycemic principle of *Mesona procumbens* and *Orthosiphon stamineus*. *T'ai-wan K'o Hsueh* **1984**, *38*, 26−31 (in Chinese).
(7) Liu, J. Pharmacology of olanolic acid and ursolic acid. *J. Ethnopharmacol.* **1995**, *49*, 57−68.
(8) Balanehru, S.; Nagarajan, B. Protective effect of oleanolic acid and ursolic acid against lipid peroxidation. *Biochem. Int.* **1991**, *24*, 981−990.
(9) Kazuki, H.; Shinichi, K.; Michio, Y. Natural antioxidants and active oxygen removers in food cosmetics, and pharmaceuticals. *Chem. Abstr.* **1996**, *124*, 200690r.
(10) Yen, G. C.; Hung, C. Y. Effects of alkaline and heat treatment on antioxidative activity and total phenolics of extracts from Hsian-tsao (*Mesona procumbens* Hemsl.). *Food Res. Int.* **2000**, *33*, 487−492.
(11) Conde, E.; Cadahia, E.; Garcia-Vallejo, M. C.; Simon, B. F. D.; Adrados, J. R. G. Low molecular weight polyphenols in cork of *Quercus suber*. *J. Agric. Food Chem.* **1997**, *45*, 2695−2700.
(12) Mitsuda, H.; Yasumoto, K.; Iwami, K. Antioxidative action of indole compounds during the autoxidation of linoleic acid. *Eiyo to Shokuryo* **1996**, *19*, 210−214.
(13) Yen, G. C.; Chen, H. Y. Relationship between antimutagenic activity and major components of varius teas. *Mutagenesis* **1996**, *11*, 37−41.
(14) Kitada, Y.; Tamase, K.; Sasaki, M.; Yamazoe, Y.; Maeda, Y.; Yamamoto, M.; Yonetani, T. Determination of L-ascorbic acid, tocopherol, carotene and chlorophyll in various teas. *Nippon Shokuhin Kyogo Gakkaishi* **1989**, *36*, 927−933.
(15) Miyake, T.; Yamamoto, K.; Osawa, T. Metabolism of antioxidant in lemon fruit (*Citrus lion* Burm. F.) by human intestinal bacteria. *J. Agric. Food Chem.* **1997**, *45*, 3738−3742.
(16) Azuma, K.; Nakayama, M.; Kosaji, M.; Ippoushi, K.; Yamaguchi, Y.; Kohata, K.; Yamauchi, Y.; Ito, H.; Higashio, H. Phenolic antioxidants from the leaves of *Corchorus olitorius* L. *J. Agric. Food Chem.* **1999**, *47*, 3963−3966.
(17) Duh, P. D.; Yen, G. C. Antioxidative activity of three herbal water extracts. *Food Chem.* **1997**, *60*, 639−645.
(18) Simonetti, P.; Pietta, P.; Testolin, G. Polyphenol content and total antioxidant potential of selected italian wines. *J. Agric. Food Chem.* **1997**, *45*, 1152−1155.
(19) Yen, G. C.; Hsieh, C. L. Antioxidant activity of extracts from Du-zhong (*Eucommia ulmoides*) toward various lipid peroxidation models in vitro. *J. Agric. Food Chem.* **1998**, *46*, 3952−3957.
(20) Zhang, H. L.; Nagatsu, A.; Okuyama, H.; Mizukami, H.; Sakakibra, J. Sequiterpene glycosides from cotton oil cake. *Phytochemistry* **1998**, *48*, 665−668.
(21) Kuo, Y. H.; Shue, M. J. New esters, 2-(4-hydroxy-3-methoxyphenyl)ethyl hexa- and octacosanoates from the leaves of *Cinnamomum reticulatum* Hay. *J. Chin. Chem. Soc.* **1991**, *38*, 65−69.
(22) Sakushima, A.; Coskun, M.; Maoka, T. Hydroxybenzoic acid from *Boreava orientalis*. *Phytochemistry* **1995**, *40*, 257−261.
(23) Uchida, M.; Nakajin, S.; Toyoshima, S.; Shinoda, M. Antioxidative effective effect of sesamol and related compounds on lipid peroxidation. *Biol. Pharm. Bull.* **1996**, *19*, 623−626.
(24) Chen, C. C.; Yu, H. J.; Ou, J. C.; Pan, T. M. Constituents of the heartwood of *Diospyros eriantha*. *J. Chin. Chem. Soc.* **1994**, *41*, 195−198.
(25) Inoshiri, S.; Sasaki, M.; Kohda, H.; Otsuka, H.; Yamasaki, K. Aromatic glycosides from *Berchemia racemosa*. *Phytochemistry* **1987**, *26*, 2811−2814.
(26) Chen, C. W.; Ho, C. T. Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *J. Agric. Food Chem.* **1997**, *45*, 2374−2378.
(27) Gadow, A. V.; Joubert, E.; Hansmann, C. F. Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), α-tocopherol, BHT, and BHA. *J. Agric. Food Chem.* **1997**, *45*, 632−638.
(28) Papadopoulos, G.; Boskou, D. Antioxidant effect of natural phenols on olive oil. *J. Am. Oil Chem. Soc.* **1991**, *68*, 669−671.
(29) Pokorny, J. Major fators affecting the autoxidation of lipids. In *Autoxidation of Unsaturated Lipids*; Chan, H. W.-S., Ed.; Academic Press: London, U.K., 1987; pp 141−206.
(30) Cuvelier, M.-E.; Richard, H.; Berset, C. Comparison of the antioxidative activity of some acid-phenols: structure−activity relationship. *Biosci., Biotechnol., Biochem.* **1992**, *56*, 324−325.
(31) Sanbongi, C.; Osakabe, N.; Natsume, M.; Takizawa, T.; Gomi, S.; Osawa, T. Antioxidative polyphenols isolated from *Theobroma cacao*. *J. Agric. Food Chem.* **1998**, *46*, 454−457.
(32) Ohnishi, M.; Morishita, H.; Iwahashi, H.; Toda, S.; Shirataki, Y.; Kimura, M.; Kido, R. Inhibitory effects of chlorogenic acids on linoleic acid peroxidation and haemolysis. *Phytochemistry* **1994**, *36*, 579−583.
(33) Hung, C. Y.; Yen, G. C. Extraction and identification of antioxidative components of Hsian-tsao (*Mesona procumbens* Hemsl.). *Food Sci. Technol.* **2001**, *34*, 306−311.

Received for review November 2, 2001. Revised manuscript received February 26, 2002. Accepted February 27, 2002. This research work was partially supported by the National Science Council, the Republic of China, under Grant NSC90-2316-B005-001.

JF011454Y